Supporting Information

Yang et al. 10.1073/pnas.1302703111

SI Materials and Methods

Animal care and experiments performed were in accordance with the protocols approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. Double-color in situ hybridization was performed with digoxigenin-labeled and fluorescein-labeled riboprobes. Antibodies directed against digoxigenin and fluorescein were conjugated with either alkaline phosphatase or horseradish peroxidase for color development with chromogenic substrates. Probes for *Col1a1, Col10a1,* and *Mmp13* were described previously (1); *LacZ* probe is a 289-bp fragment of *LacZ*, and *Cre* probe is a gift from Takahiro Ohyama (University of Southern California, Los Angeles). The following antibodies were obtained commercially: osterix (ab22552; Abcam), type I collagen (ab34710; Abcam), sclerostin (AF1589; R&D Systems), red fluorescent protein (ab62341; Abcam), and GFP (ab6556; Abcam). Reporter mouse strains RLacZ (2), RYFP (3), Z/EG (4), and RtdTomato (5) were reported before.

1. Rosati R, et al. (1994) Normal long bone growth and development in type X collagen-null mice. *Nat Genet* 8(2):129–135.
2. Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21(1):70–71.
3. Srinivas S, et al. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4.
4. Novak A, Guo C, Yang W, Nagy A, Lobe CG (2000) Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. *Genesis* 28(3-4):147–155.
5. Madisen L, et al. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133–140.

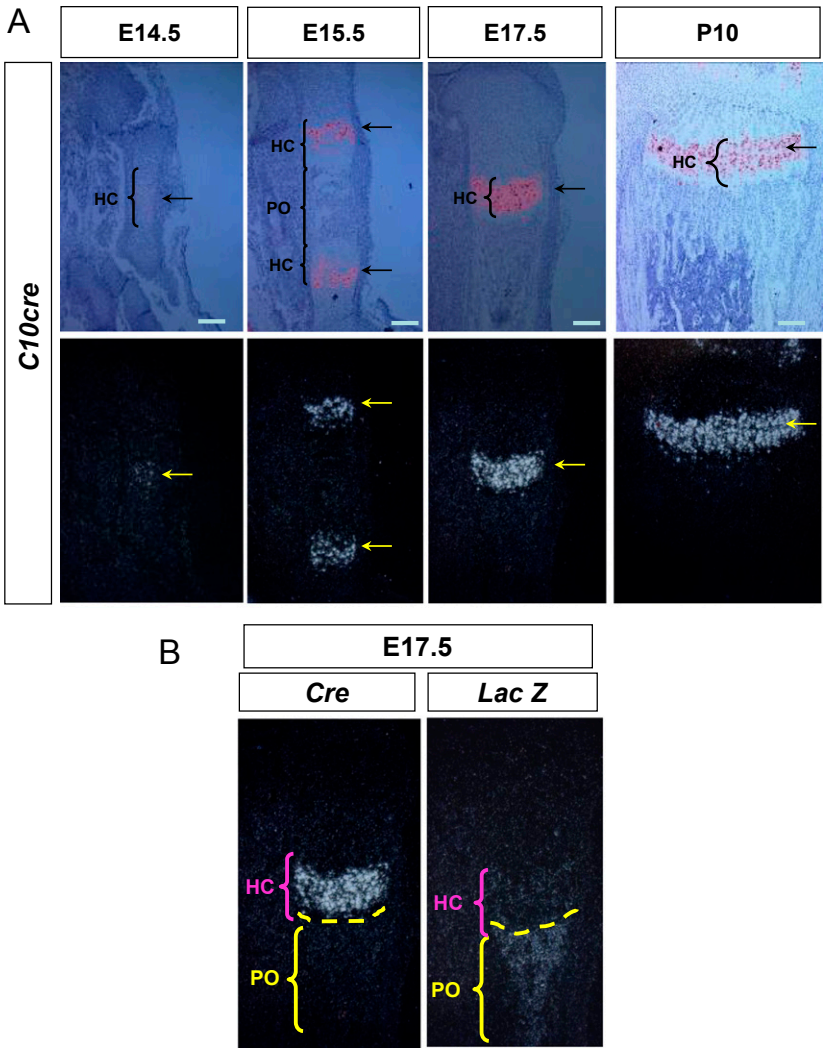

Fig. S1. Hypertrophic chondrocyte (HC)-specific expression of *Col10a1-Cre* and subsequent induction of LacZ expression in HC-derived cells in the primary ossification center (POC). (*A*) During tibia development, chondrocytes in the center of the cartilage template started to hypertrophy and *Cre* expression was detected by in situ hybridization at embryonic day 14.5 (E14.5; upper panels show a composite of bright-field and dark-field images in the lower panels). Afterward, *Cre* mRNA was specifically expressed by the HCs and down-regulated in the lower hypertrophic zone. No Cre-expressing cell was found in POCs at E15.5 and E17.5. (Scale bar, 100 mm.) (*B*) At E17.5, the expression of *Cre* gene was limited to HCs. However, *LacZ* transcript not only was observed in HCs, but also was present in cells in the POC, indicating they are HC-derived cells. HC, zone of HCs; LH, zone of late HCs; PO, primary ossification center.

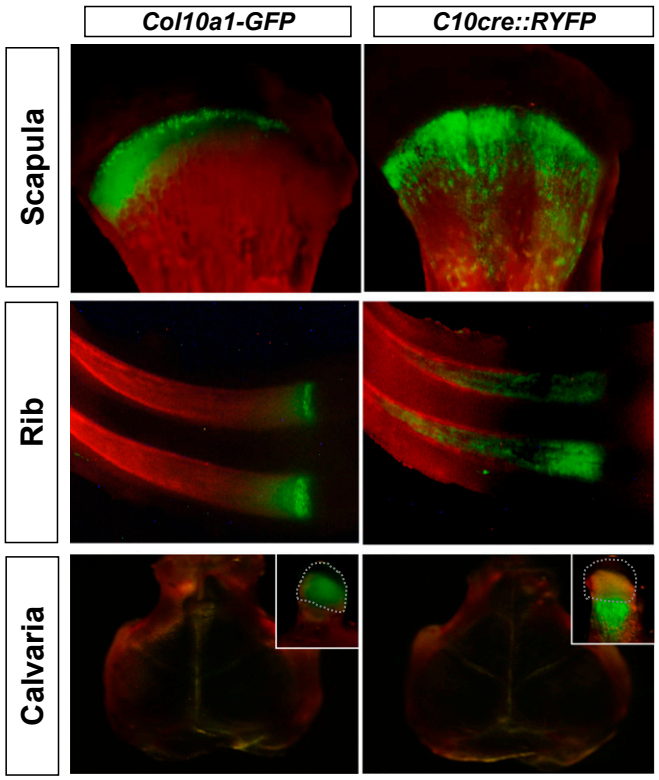

Fig. S2. Presence of HC-derived cells in endochondral but not intramembranous bones. *Col10a1-GFP*–expressing cells were present in the hypertrophic zone of postnatal day 10 (P10) endochondral bones, such as the scapula and ribs, but were not found in intramembranous bones of the calvaria. Similar results were obtained for *C10Cre::RYFP* mice, which mainly display the presence of fluorescent HC-derived cells in trabecular bone regions of the endochondral bones. (*Inset*) Fluorescent HCs in the mandibular condyle, which forms by endochondral ossification. The dotted line delimits the cartilage end of the condyle. (*Left*) *Col10a1-GFP* mice; (*Right*) *C10Cre::RYFP* mice.

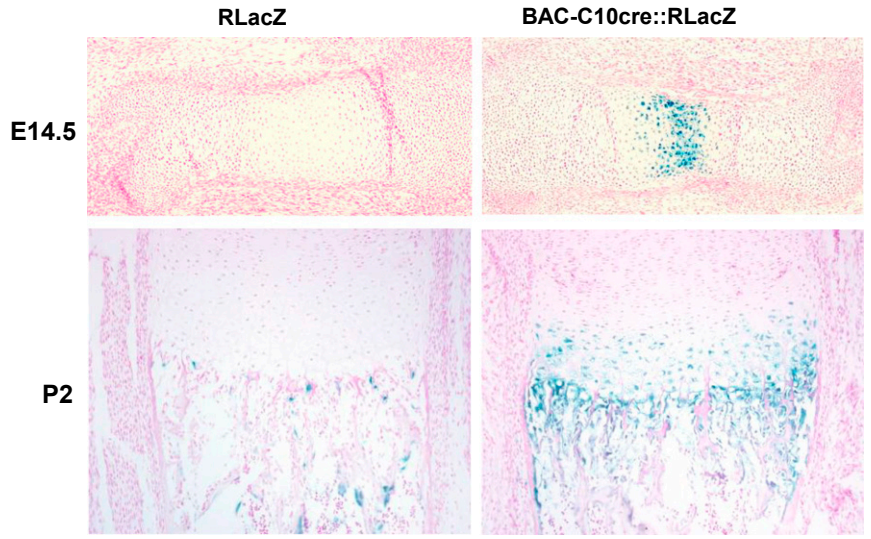

Fig. S3. Transgenic BAC-C10Cre activates LacZ reporter in HCs and HC-derived cells in the trabecular bone region. We have generated *BAC-C10Cre* transgene from the BAC clone RPCI23-194I3, which carries the *Col10a1* locus, and we inserted *Cre* at the ATG codon in exon 2. Included in the transgene were 154 kb and 35 kb of 5′ and 3′ sequences, respectively, relative to *Col10a1* exons. A BAC-C10Cre transgenic mouse was generated by pronuclear microinjection. Double-transgenic *BAC-C10Cre::RLacZ* mice exhibit the same X-Gal staining results as those of *C10Cre::RLacZ*, specifically labeling HCs and HC descendants in the trabecular bone region. Note that nonspecific X-Gal–stained osteoclasts were found in the control.

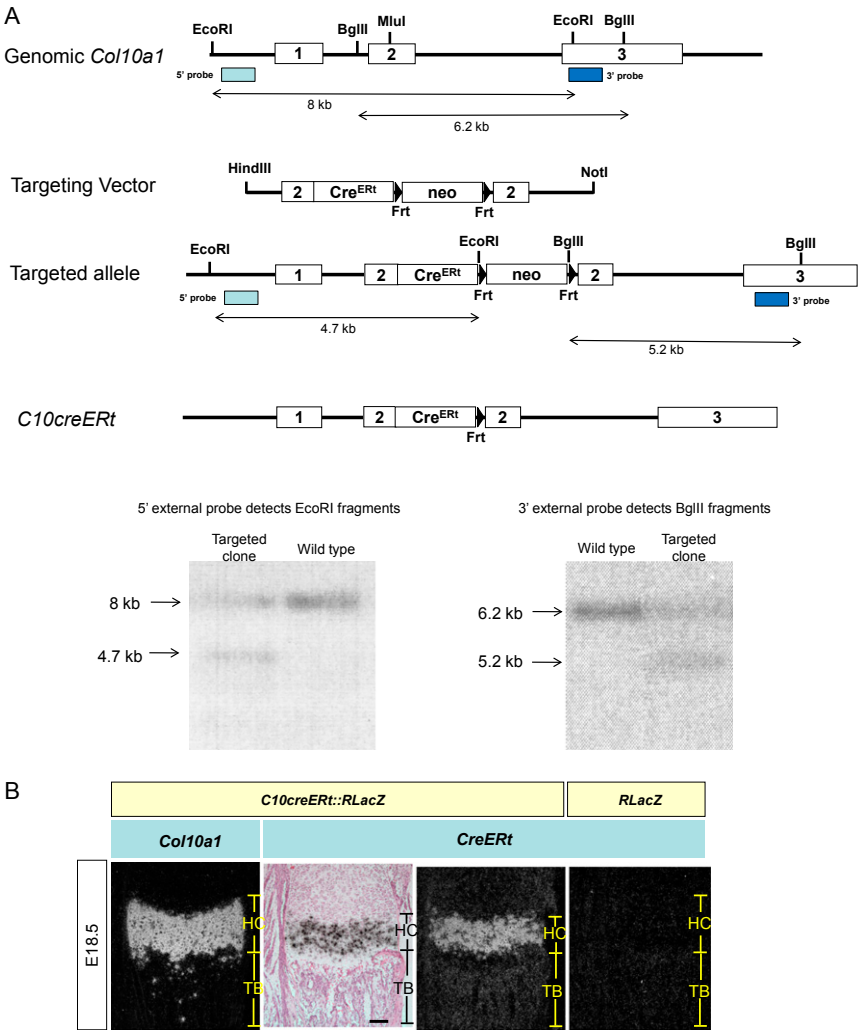

Fig. S4. Generation of inducible *Col10a1-CreERt* knock-in mice and specific *C10CreERt* expression in HCs. (*A*) A fusion gene of Cre recombinase and mutated estrogen receptor was inserted at the ATG codon in exon 2 of the *Col10a1* locus together with a neo selection cassette, which then was removed by Flp recombinase (*C10CreERt)*. Correctly targeted ES cell clones were identified by Southern blot analyses using external probes for the 5′ end and 3′ ends, which detect, respectively, EcoRI and BglII fragments. Wild-type ES cells contain only the *Col10a1* allele, whereas the targeted ES cells contain one *Col10a1* and one targeted allele. (*B*) In situ hybridization for *Col10a1* and *CreERt* expression in tibia sections of *C10CreERt::RLacZ* and *RLacZ* control mice at E18.5. CreERt is exclusively expressed in HCs. (Scale bar, 100 mm.) HC, zone of HCs; PO, primary ossification center; TB, trabecular region.

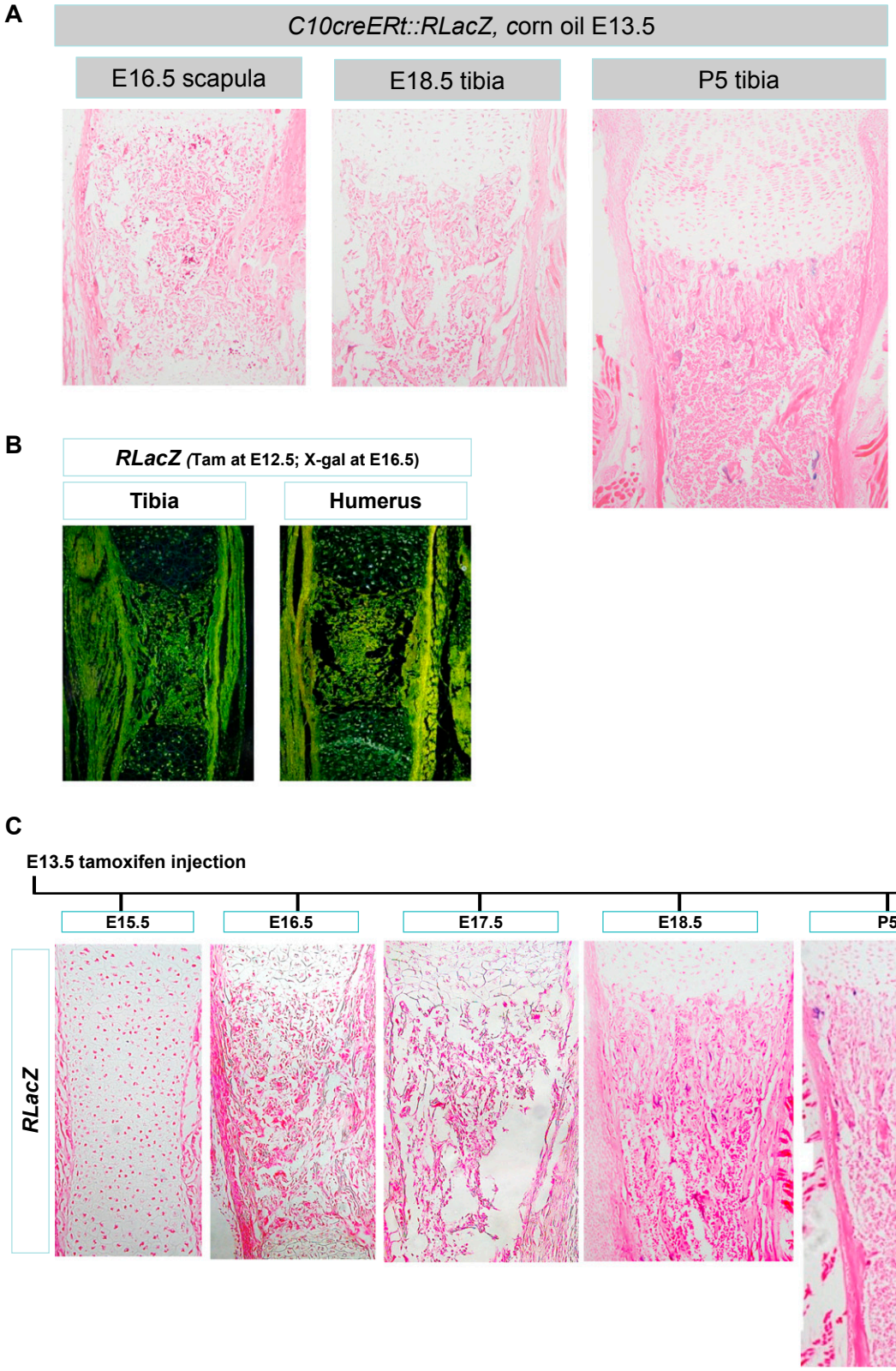

Fig. S5. Control X-Gal staining indicates specific tamoxifen (tam)-induced *Col10a1-CreERt* activity. (*A*) When injected with corn oil only without tam at E13.5 followed by 6-h X-Gal staining, nonspecific X-Gal staining was negligible in E16.5, E18.5, and P5 *C10CreERt::RLacZ* bones. (*B*) No $LacZ^+$ cell was detected in control tibia and humerus at E16.5 after tam injection at E12.5. (*C*) No $LacZ^+$ cell was detected in control tibiae up to E17.5 after tam injection at E13.5. A few $LacZ^+$ osteoclasts could be detected at E18.5 and P5, and apparently more $LacZ^+$ osteoclasts were detected at P1month (P1m), but no $LacZ^+$ osteocytes could be found in control (*Inset*).

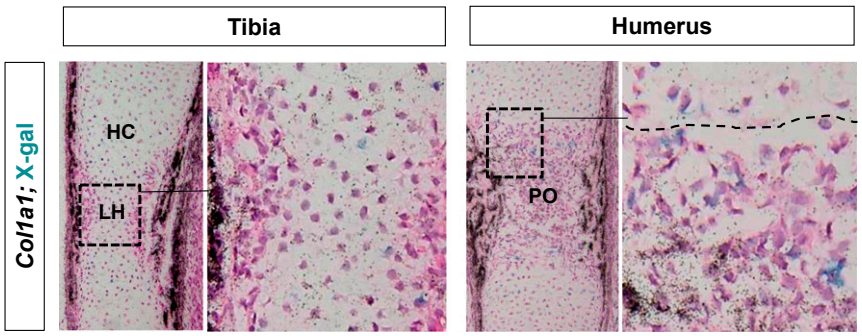

Fig. S6. Absence of *Col1a1* expression in LacZ^{+} HC-derived cells 24 h after tam injection at E14.5. In the same set of experiments as in Fig. 2*E*, *Col1a1* in situ hybridization signal did not colocalize with LacZ^{+} cells in the POC of humerus, suggesting HC-to-osteoblast transition takes more than 24 h to accomplish. The dotted line denotes the chondro-osseous junction in the humerus. HC, zone of HCs; LH, zone of late HCs; PO, primary ossification center.

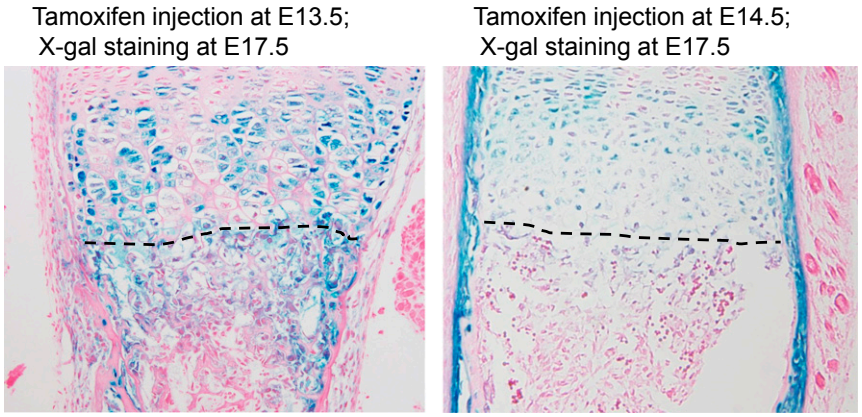

Fig. S7. Presence of LacZ^{+} cells in the POC of *Col2CreERt::RLacZ*. Tam was injected at E13.5 (*Left*) or E14.5 (*Right*) to a pregnant mouse, and X-Gal staining was performed at E17.5. (*Left*) LacZ^{+} cells can be seen clearly in the trabecular bone region of the proximal femur of the *Col2CreERt::RLacZ* fetus. (*Right*) In contrast, fewer LacZ^{+} can be found in this panel, and the perichondrium and periosteum are strongly stained. Dotted lines denote the chondro-osseous junction.

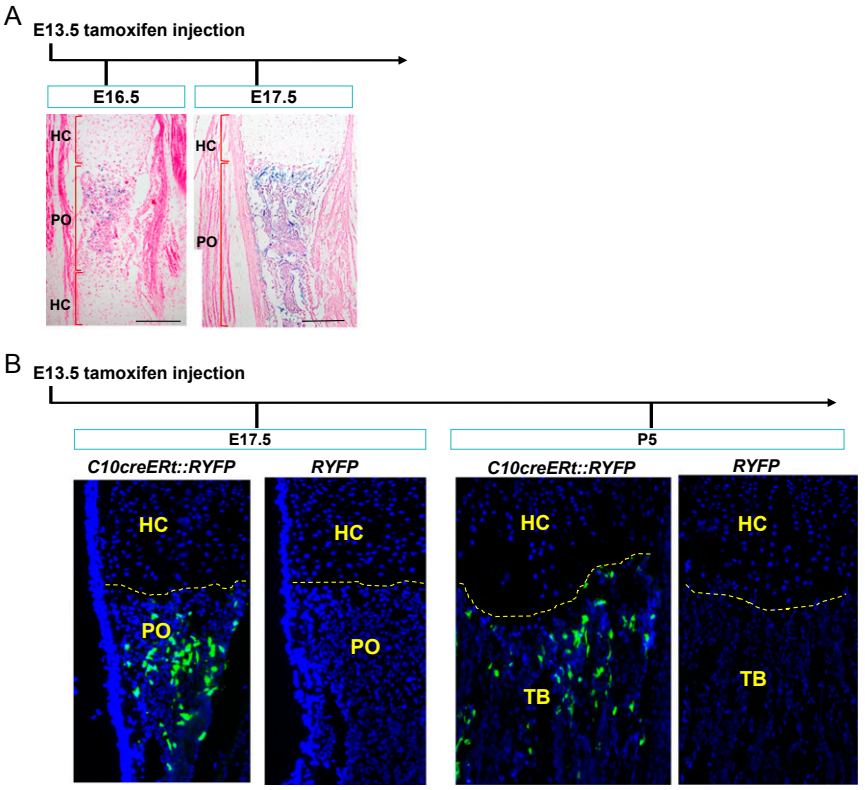

Fig. S8. Presence of HC descendants in the POC of *C10CreERt::RLacZ/RYFP* mice. (*A*) X-Gal staining of *C10creERt::RLacZ* tibia sections at E16.5 and E17.5 after tam injection at E13.5. (*B*) Anti-YFP antibody staining of *C10CreERt::RYFP* tibia cryosections at prenatal (E17.5) and postnatal stages (P5) after tam injection at E13.5, in parallel to the results shown in Fig. 3*A*. No YFP^+ cell was found in controls.